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Laboratory validation of a new gas-enhanced dentine liquid permeation evaluation system

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Abstract: AIM: To validate a new automated dentine permeability testing platform based on pressure change measurements. **METHODOLOGY:** A split chamber was designed allowing for concomitant measurement of fluid permeation and pressure difference. In a first test, system reliability was assessed by interposing a solid metal disk, embedded composite resin disks, or teeth by consecutively measuring eight times under standardized conditions. Secondly, the repeatability and applicability of the method was tested in a dentine wound model by using intact third molars: Class I (2×5 mm) and a full occlusal preparation as well a ceramic restoration were consecutively performed and repeatedly measured eight times each. In the last test, the system detection limit as well correlation between gas pressure difference and liquid permeation were evaluated: Again, third molars were used and occlusal preparations of increasing size (2×5 , 3×5 , 4×5 , and 5×5 mm and full occlusal preparations, respectively) were made. Data was analyzed for the linearity of measurement, and R (2) values were calculated. **RESULTS:** The embedding procedure allowed for perfect separation of the two chambers, and no significant variation in repeated measurements of evaluated samples for the respective treatments ($p = 0.05$) was found. The detection was 0.002 hPa/min for the pressure slope and 0.0225 l/min for the fluid infiltration, respectively. The saline volume was highly correlating to the gas pressure changes ($R(2) = 0.996$, $p < 0.0001$). **CONCLUSIONS:** The presented method is a reliable and exact tool to assess dentine permeability by nondestructive and repeatable measurements. **CLINICAL RELEVANCE:** This method is suitable for measurements and comparison of the effectiveness of dentine wounds sealing materials.

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Laboratory validation of a new gas-enhanced dentine liquid permeation evaluation system

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Laboratory validation of a new gas-enhanced dentine liquid permeation evaluation system

Abstract

Aim: To validate a new automated dentine permeability testing platform based on pressure change measurements.

Methodology: A split-chamber was designed allowing for concomitant measurement of fluid permeation and pressure difference. In a first test, system reliability was assessed by interposing a solid metal disc, embedded composite resin discs, or teeth by consecutively measuring eight times under standardized conditions. Secondly, the repeatability and applicability of the method was tested in a dentine wound model by using intact third molars: Class I (2×5mm) and a full occlusal preparation as well a ceramic restoration were consecutively performed and repeatedly measured 8 times each. In the last test, the system detection limit as well correlation between gas pressure difference and liquid permeation were evaluated: Again, third molars were used and occlusal preparations of increasing size (2×5,3×5,4×5,5×5mm and full occlusal preparations, respectively) were made. Data was analyzed for the linearity of measurement and R^2 values were calculated.

Results: The embedding procedure allowed for perfect separation of the two chambers and no significant variation in repeated measurements of evaluated samples for the respective treatments ($p=0.05$) was found. The detection was 0.002 hPa/min for the pressure slope and 0.0225 $\mu\text{l}/\text{min}$ for the fluid infiltration, respectively. The saline volume was highly correlating to the gas pressure changes ($R^2=0.996$, $p<0.0001$).

Conclusions: The presented method is reliable, and exact tool to assess dentine permeability by non-destructive and repeatable measurements.

Clinical Relevance: This method is suitable for measurements and comparison of dentine wounds sealing materials effectiveness.

Key words: Dentine, sealability, permeability, restoration leakage.

1. Introduction

The unique tubular structure of dentine is mainly related to evolutionar functional adaptation to enable mastication by transducing bite pressures into tensile forces in the collagen matrix [1]. In addition, fluid-filled dentinal tubules allow for transducing stimuli to the underlying pulp [2]. This results in a sophisticated functional and sensitive organ. On the other hand, exposed dentinal tubules can lead to dentine hypersensitivity or – if adjacent to infectious processes - reflect pathological conditions like caries [3]. Effective protection of dentinal tubules has therefore a pivotal role in clinical dentistry.

After the observation that fluids could permeate through dentinal tubules of extracted teeth [4,2], various *in vitro* models were established to study dentine wounds and were modified later to test for leakage in restorations and root canal fillings. The versatile split-chamber model to test infiltration of isotopes was revolutionary in that field [5]. It had a simple design to hold and test small dentinal disc specimens. A decade later, Derkson and co-workers introduced – inspired by the fluid shift model of Brännstrom - their pressurized fluid transport model, which aimed to test the seal around restorative fillings [6]. The same set-up was adapted to test the seal of root canal fillings [7]. The fluid shift model was later digitized to measure the infiltrated fluid volume in real time [8]. In 2008, Romieu and co-workers [9] introduced a new dimension in leakage measurements using a testing system with two pressurized chambers. By continuously recording the air pressure difference between the two differently pressurized chambers, the ratio of pressure change provided an indirect value of air leakage. However, this evaluation was performed under dry conditions, which may be considered a significant shortcoming of this method and potentially results in dehydrated test specimens and an unrealistic simulation with regard to the originally intended oral cavity conditions to be tested.

Since the hydrodynamic theory is widely accepted to explain dentine sensitivity [10], the fluid infiltration method may still be considered as the gold standard in permeability/leakage testing and it can be adopted to many types of leakage testing. However, most of these currently available testing models exhibit some disadvantages. Among them, the long testing time, the difficulty of establishing a repeatable set-up, the lack of internal control and possible entrapment or reaction of perfusing substances with the sample are worth mentioning. Another potential bias,

which was underestimated for a long time was the permanent fixation in adhesive materials (epoxy resins, waxes, etc.) without adequate testing before and after treatment, which resulted in a lack of an internal quality control. Therefore - not surprisingly - it has been shown that these embedding processes can also lead to potential overestimation in permeation testing [11]. Another disadvantage of most set-ups, namely to test only at a single time, additionally limits the possibility to compare between different treatments or even the same treatment at different stages using the same specimen.

Due to these limitations, a new testing platform was designed aiming to reliably measure sealability based on a combination of previously mentioned models, namely a split-chamber model measuring fluid permeation and the resulting gas pressure difference simultaneously. The aim of this study was to validate the accuracy as well the leakage-free embedding of samples. Reproducibility of repeated measurements was assessed. The working hypotheses and requirements were as follows:

- 1) The embedding causes no false-positive measurements.
- 2) The repeated measurements of identical samples results in reproducible results.
- 3) The detection limit to assess permeation is low.
- 4) The liquid collected during the permeation test correlates to the gas pressure differences.

2. Materials and methods

2.1. Set-up of the leakage/permeability measuring device

The split testing chamber model consisted of two custom-made plexiglass parts, which were tightened together using three solid screws (Fig 1). The embedded specimens were fixed between the two parts using a rubber O-ring with an outer diameter of 22 mm, an inner diameter of 15 mm and a thickness of 3.5 mm, thus forming two fully separated chambers with the sample fixated in between. The lower chamber was opened at its lower terminal with an adapter fixed to the outside allowing the placement of an eppendorf tube to collect the permeating liquid. The two chambers were connected to two valves to stabilize their pressure during testing once they were closed.

The temperature was controlled as follows: The permeability/leakage unit (Fig 2, a) was installed in an isolation chamber (Fig 2, b), in which the temperature was constantly held at 35°C. This chamber was situated in a second larger experimental box (Fig 2 c), in which the temperature was always kept at 31°C. The room temperature was stable at 25°C.

2.2 Pressure difference measurements

A pressure difference measuring device (Testo 526, Testo AG, Lenzkirch, Germany) was connected by its two inlets to the tubes connected to the upper and lower chambers just before the valves, which allowed for real-time measurement. The measuring device was connected to a computer-unit running a proprietary program (V 4.2 SP2, Testo AG, Germany). The O-ring was lubricated with a silicon grease (Molykote 111 compound, DOW Corning GMBH, Germany) to improve the sealability between the two chambers. The specimen was then positioned in the ring, and 2.5 ml of a pre-pressurized (N₂ gas 860 hPa) 0.9% NaCl solution was added on top in the upper chamber. The cover was repositioned and the three screws were tightened using a torque-controlled screwdriver. The upper chamber was then pressurized with N₂ gas to 860 hPa. The lower chamber was negatively pressurized down to minus 170 hPa. This resulted in an effective pressure difference of 1030 hPa between the two chambers. Given the hypothesis that there is a connection between the two chambers, i.e. leakage through the sample, this would affect the pressure difference. The pressure difference would change and become smaller by penetration of the NaCl solution from the positive pressure chamber to the low pressurized chamber causing a pressure drop in the positive side and a pressure increase in the negative side, until the pressure is equalized in both chambers and the difference reaches to 0 hPa. The pressure difference measurements were started and continued for 40 min at a rate of 1 measurement/sec. The reading resulted in a data set and a curve representing the rate of pressure change expressed as a drop in pressure difference over time. The pressure value at two fixed time points (1200 sec and 2400 sec) were defined to calculate the slope in between:

$$\text{Slope} = \frac{P_2 - P_1}{T_2 - T_1} \text{ hPa/min.}$$
 All results were expressed as positive values for the statistical analysis for the ease of understanding, as we aimed to show a positive correlation with the infiltrated fluid volume.

These optimal time points to detect the slope were found by preliminary observations on repeated measurements of the same sample to be reproducible (data not shown). In addition, the infiltrated physiological saline solution was collected and weighed to calculate the volume that permeated the specimen (see below, 2.5).

2.3 Specimen preparation

To test the tightness/sealability, repeatability, detection limit, correlation between the measured outcomes and the capability of the embedding procedures in maintaining a tight seal after multiple measurements with no or minimal changes, a solid metal disc, embedded composite discs, and third molars were interposed. The solid metal disc (3 mm thick and had a diameter of 15 mm) was chosen as gold standard for tightness, as no embedding procedure was involved, and thus no additional interfaces were created. The solid metal disc had the exact thickness and outer dimensions of the embedding brass rings used in the set-up (Fig 4, Exp. A). It was used to measure the internal system leakage at all joints and connections. Therefore – hypothetically - this test should result in no leakage and served as an internal system tightness control.

The round composite discs had a diameter of 7 mm and a thickness of 3 mm and were fabricated using a teflon mold and composed of dual cure composite build-up material (Luxa Core Automix, DMG, Hamburg, Germany). This allowed for the formation of a non-porous solid biomaterial/tooth surrogate sample given the hypothesis that no leakage should occur given adequate sealing around it. Accordingly, third molars were selected as natural products from the department's pool of extracted teeth. They were extracted for reasons not related to the current study from patients aged 18-20 yrs. All teeth were free of caries and restorations. The roots were not fully developed ensuring proper pass to the pulp chamber and allowing for retrograde pulp extirpation. Samples were stored in 0.2% thymol at a temperature of 5°C for no longer than one year. Both, composite discs and teeth, were embedded in custom-made brass rings with an outer diameter of 15 mm, an inner counterpart of 10 mm, and a thickness of 3 mm. The rings were sandblasted on their inner surface using 50-µm aluminium oxide (Benzer-Dental AG, Zurich, Switzerland) and the specimens were embeded using a light-curing nail build-up material kit (Sina, Shenzhen Cyber Technology Ltd, Mainland, China). This material consisted of a primer, a gel, and a glaze material. The teeth as well the rings were primed and light-cured for 2 minutes in a lightcure chamber (Spectramat, Ivoclar-Vivadent, Schaan, Liechtenstein). Subsequently, the parts were fixed in position using a rubber carrier made of a putty material material (Optosil, Heraeus Kulzer GmbH, Hanau, Germany) (Fig 3). The gel was applied in one increment to fill the space between the ring and sample. Care was taken not to allow excess material formation on the two upper or lower surfaces of the metal ring. The build-up was then light cured for 4 minutes. Finally, the glaze layer was applied to the surface to eliminate any imperfections in the embedding gel buildup, which was finally light cured for another 4 minutes. This embedding method was used for all repeatability and correlation samples tested as described in this study.

2.4 Sealing accuracy and repeatability evaluation

The metal and the composite discs as well 3 intact third molars were prepared as described above and pressure difference measurements were repeated 8 times each (Fig 4, a) to establish an initial reference baseline.

In addition, three third molar teeth were measured for permeability after creation of dentine wounds (class I preparations; 2×5 mm and a depth of 2 mm from the fissure level) and a subsequent full occlusal surface preparation, which completely removed the occlusal enamel until the CI preparation floor. All preparations were made using a tapered diamond bur (Number 8117, Intensiv SA, Montagnola, Switzerland) attached to a parallell drill holder (Cendres & Metaux SA, Biel, Switzerland). To ensure no effect of the repeated measurements on the embedding, the teeth then were restored after conditioning (Clearfil SE Protect, Kuraray America Inc., USA) according to the manufacturer's instructions using CAD/CAM onlays (Sirona Cerec Blocs, VITA Zahnfabric, Bad Säckingen, Germany) cemented with Multilink (Ivoclar Vivadent AG, Liechtenstein). Again, all samples were tested eight times (Fig. 5, Exp. A&B). The different measurements for each sample for the respective treatments were carried out on different days to assess potential influence of storage on the embedding and permeability. For the interim, samples were kept in physiologic saline at room temperature.

2.5 System detection limit and correlation between pressure difference and fluid permeation

To assess the correlation between the two quantitative primary outcome parameters of the device, i.e. gas pressure difference change and liquid permeation, 6 additional third molar teeth from the department's collection of extracted teeth were used (molars 4-9). They were tested after embedding and before preparation to assess the baseline performance, i.e. tightness. The measured curves were used to determine the method detection limit, i.e. the minimum measured permeability value that could be observed in a sample with confidence. Subsequently, consecutive preparations were performed in all specimens with increasing invasivity and dimensions (2×5, 3×5, 4×5 and 5×5 mm and a depth of 2 mm from the fissure level) and finally a full occlusal trimming was performed as described under section 2.4 (Fig. 5, Exp. C). After each step, the pressure difference change was measured as described above (Fig 4, b). In addition, the saline that permeated each specimen was collected in the tube that was attached to the apparatus.

The volume of liquid was measured by calculating the weight difference of the tube before and after the experiment using a precision scale (Mettler AT261 Delta Range, Greifensee, Switzerland).

2.6 Data presentation and analysis

Repeatability of the individual pressure change difference within the same sample for the same treatment was assessed using a linear mixed model.

Permeability expressed as the slope in hPa/min and the total permeating water volume were calculated separately for each of the four conditions (baseline after embedding, CI I preparation, full occlusal preparation and restoration) and results were presented as the range of data obtained in the individual measurements (original measurement and 7 repetitions).

To assess the detection limit, the measurement background noise in the test curves of the sound 6 teeth at fixed 9 time points with 120 seconds intervals was calculated mathematically. It was calculated by measuring the deviation from the ideal curve drawn between the two fixed time points to determine the leakage slope value independently. When the ideal slope value (hPa/min) and the time interval are known, it is possible to calculate the ideal measurement value at each point. The deviation from this was calculated, and average deviations were then pooled for each sample and used for further calculations [12].

To test whether the slope in the pressure change over time correlated with the collected saline solution ($N = 6$), the Pearson correlation coefficient was used [13] (Fig 5).

3. Results

The mean slope values (Table 1) for the baseline measurements, i.e. the measurements of the sample permeability status before treatment, ranged between 0.01-0.03 hPa/min, indicating proper embedding seal of the specimens. The range of variation after repeated measurements of a sample did not exceed the 0.01 hPa/min. Testing for repeatability, a high linearity was shown (Table 2), indicating consistent results obtained with specimens that were measured multiple times.

The detection limit of 0.043 hPa for the pressure difference was calculated, which correlated to a slope value of 0.002 hPa/min and a fluid infiltration of 0.0225 $\mu\text{l}/\text{min}$. Testing for the pressure difference- infiltrated fluid volume correlation using the Pearson coefficient with the confidence interval set at ($p=0.05$) showed the point estimate of 0.99785 with standard deviation of

0.0002387463 ($R^2=0.996$). This confirmed the high correlation between pressure change and fluid filtration (Fig 5).

Consequently, all four working hypotheses were accepted.

4. Discussion

Permeation testing methods varied over the last years with many modifications, however, most models focussed on fluid infiltration [2]. The variance in methodology, unfortunately, still makes it difficult to interpret and compare results. Therefore, leakage testing is not any more unambiguously accepted in some scientific journals due to the fact that it cannot be ensured that leakage measured is related to actual treatment status only [14]. Permeation might also occur through other niches leading to false positive results. This study therefore tried to establish and validate a novel device to test dentin permeability more reliably under standardized conditions. The focus of this study was basically to assess the accuracy of the combined determination of fluid permeation and pressure changes over time as well as the leakage-free embedding of samples and the reproducibility of their repeated measurements, which altogether build the basis for any kind of evaluation using this device in the future.

The presented set-up is a non-destructive technique allowing testing under environmental conditions because of the use of a net effective pressure 1030 hPa close to atmospheric air pressure under a standardized simulated mouth temperature of 35° [15]. The repeatability and accuracy can be related to the standardized conditions.

This study showed that the embedding procedure allowed for perfect separation of the two chambers and that not only a very minute variation in repeated measurements of the evaluated samples for all treatments was found. In addition, the saline volume was highly correlating to gas pressure changes with a low detection limit. Therefore, the presented method appears to be a fast, reliable, and exact tool to assess permeability allowing for non-destructive and repeatable leakage measurements.

Unlike previous methods, the embedding procedure for each sample was tested independently. This allowed for the baseline status to be considered once the effective permeability is calculated for. Under normal conditions, embedding seal depends on the researcher skills and the material used. Technically, this does not play a major role in the current set-up, as it will be compensated for. The embedding material used was chosen after long trials with other materials. Waxes proved not to be sealing properly, especially with teeth. Epoxy glue resins were also screened:

Although they were initially tight, they could not withstand storage conditions in liquids for more than 24 hours, while composite resin materials had problems to stick to brass and silicon and were not adequately sealing in thin sections. The only material found to last after long storage in liquids and multiple measurements with a proper adherence to all mounted parts was a simple nail build-up gel as presented. Although the embedding base line measurement varied slightly among the samples, the possible false positive error was overcome by subtracting the base line slope value from the subsequent measurements to calculate for the absolute permeation value.

The testing under standardized temperature is usually also an ignored aspect [16], In many studies testing was done at room temperature [10]. The system allowed testing under moisturized conditions and a temperature of 35°, which is the average temperature in the oral cavity [15]. The need for testing at this constant temperature is important, as it was demonstrated that dentine permeability increases with higher temperatures [16,17]. Unlike pure gas testing units and porometers, the device prevents sample dehydration, which allows for further testing of the same samples without affecting their physical properties. Another reason to simultaneously apply pressure and vacuum is the wish to eliminate any bubble entrapment which might interfere with the permeability testing as it is the case in passive permeation testing [18],

In addition, the new chamber design and embedding makes it easy to re-mount specimens for consecutive testing, which overcomes the problem of calibrating the air bubble in position, a problem encountered in the latter method. In addition, the simple small carrier system opens the door for multiple steps and interventional studies using the same sample in different conditions to produce comparative data for proper conclusions. This contrasts with substance permeation methods, in which the results depend on the permeated substrate molecular size, osmolarity and possible capability of entrapment or reacting with other substrates in the tested samples, and as an end effect might result of under estimation of the real permeability status of the specimen. The current set-up overcomes these shortcomings by using a physiologic saline solution, which does not have any interaction or interference with the permeation process.

While validating the new method, a strong evidence of accuracy and repeatability in correlation to the permeated fluid volume for both biological as well artificial samples was found. Therefore, this method appears suitable for longitudinal in vitro studies with repeated measurements in the dental field. Although there was a slight deviation from 0, the correlation was high. This can be explained either by the difficulty in collecting some entrapped fluids in the sample, or possible evaporation under low pressurized conditions. The ease of embedding

process and mounting of samples reduced the effort during the testing procedure, the samples pre-testing before treatments ensured the compensation for the error related to the baseline status of the sample.

Conclusion

The embedding causes no false-positive measurements and the chamber model per se is tightly sealed.

- 1) The repeated measurement of identical samples results in reproducible results.
- 2) The detection limit to assess permeation is low.
- 3) The liquid collected during the permeation test correlates to the gas pressure differences.

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Conflict of interest:

The authors declare that they have no conflict of interest.

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Tables:

Table 1 – Slopes of regression lines according to respective specimen

Specimen	Initial hPa/min	Class 1 preparation hPa/min	Occlusal full preparation hPa/min	After restoration hPa/min
Metal disc	0.01 (0.01, 0.01)	-	-	-
Composite disc 1	0.02 (0.02, 0.02)	-	-	-
Composite disc 2	0.03 (0.03, 0.03)	-	-	-
Composite disc 3	0.02 (0.02, 0.03)	-	-	-
Third molar 1	0.02 (0.02, 0.02)	0.19 (0.19, 0.19)	0.36 (0.36, 0.36)	0.02 (0.02, 0.03)
Third molar 2	0.02 (0.02, 0.02)	0.21 (0.21, 0.22)	0.42 (0.42, 0.42)	0.02 (0.02, 0.02)
Third molar 3	0.03 (0.03, 0.03)	0.23 (0.22, 0.23)	0.48 (0.48, 0.48)	0.03 (0.03, 0.04)
Values indicate means and ranges (in parentheses) of 8 individual experiments.				

Table 2 – Pearson correlation coefficient values for permeation slope values and total water volume, for repeated measurements after different treatments

Variable	Initial	Class 1 preparation	Occlusal full preparation	After restoration
Slope	0.9863832	0.9964485	0.999631	0.9814998
Fluid Volume	1	0.9907555	0.9992009	1

Figure 1:

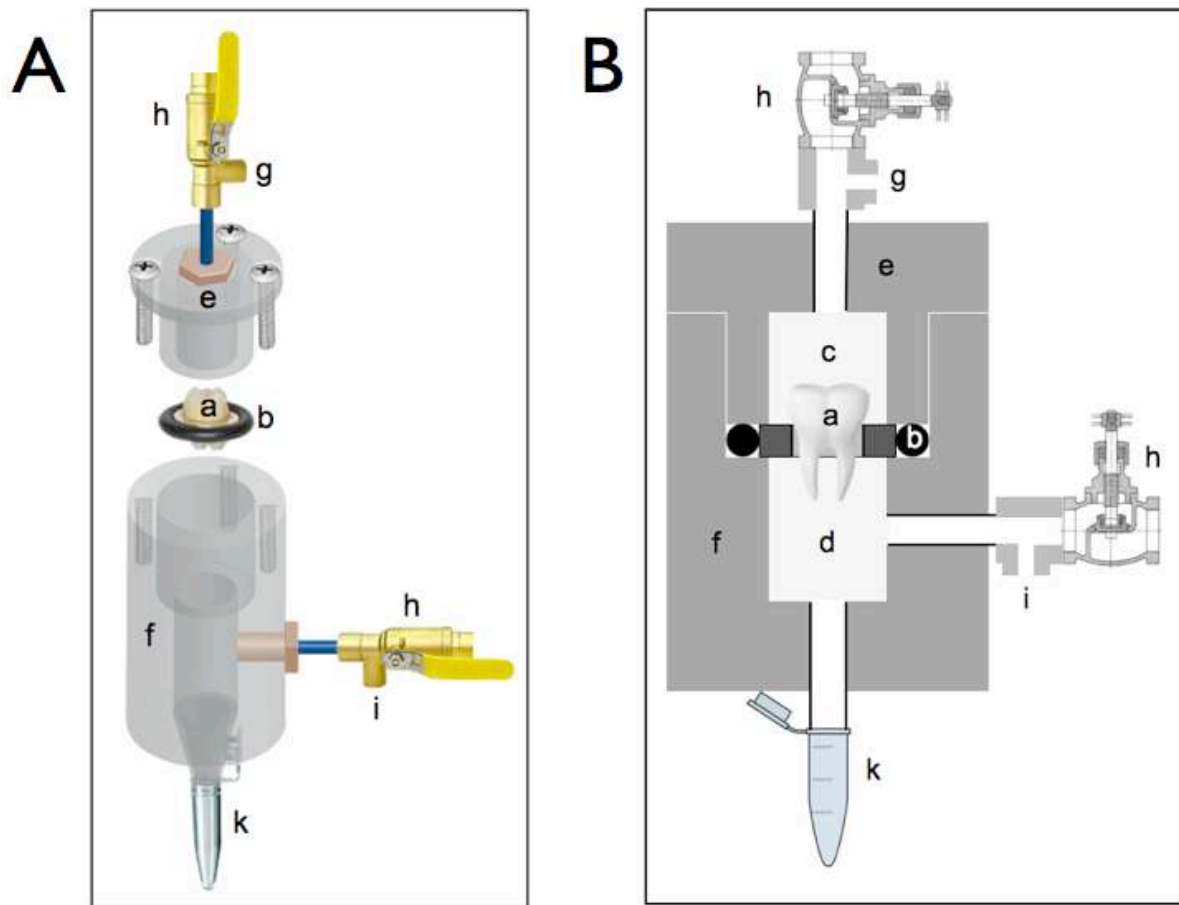


Fig 1: Split chamber with the two valves connected to control pressure on both sides

Exp. A: 3D graph, Exp. B: enhanced schematic drawing showing the position of the mounted tooth in testing. The parts are matched in both drawings. (a) A tooth sample mounted in a disc carrier. (b) O-Ring. (c) Positive pressurized chamber. (d) Low pressurized chamber. (e) Split chamber cover. (f) Split chamber body. (g) Positive outlet attached to the pressure difference measuring device. (h) Securing valves. (i) Negative outlet attached to the pressure difference measuring device. (k) Eppendorf tube to collect permeating fluid.

Figure 2:

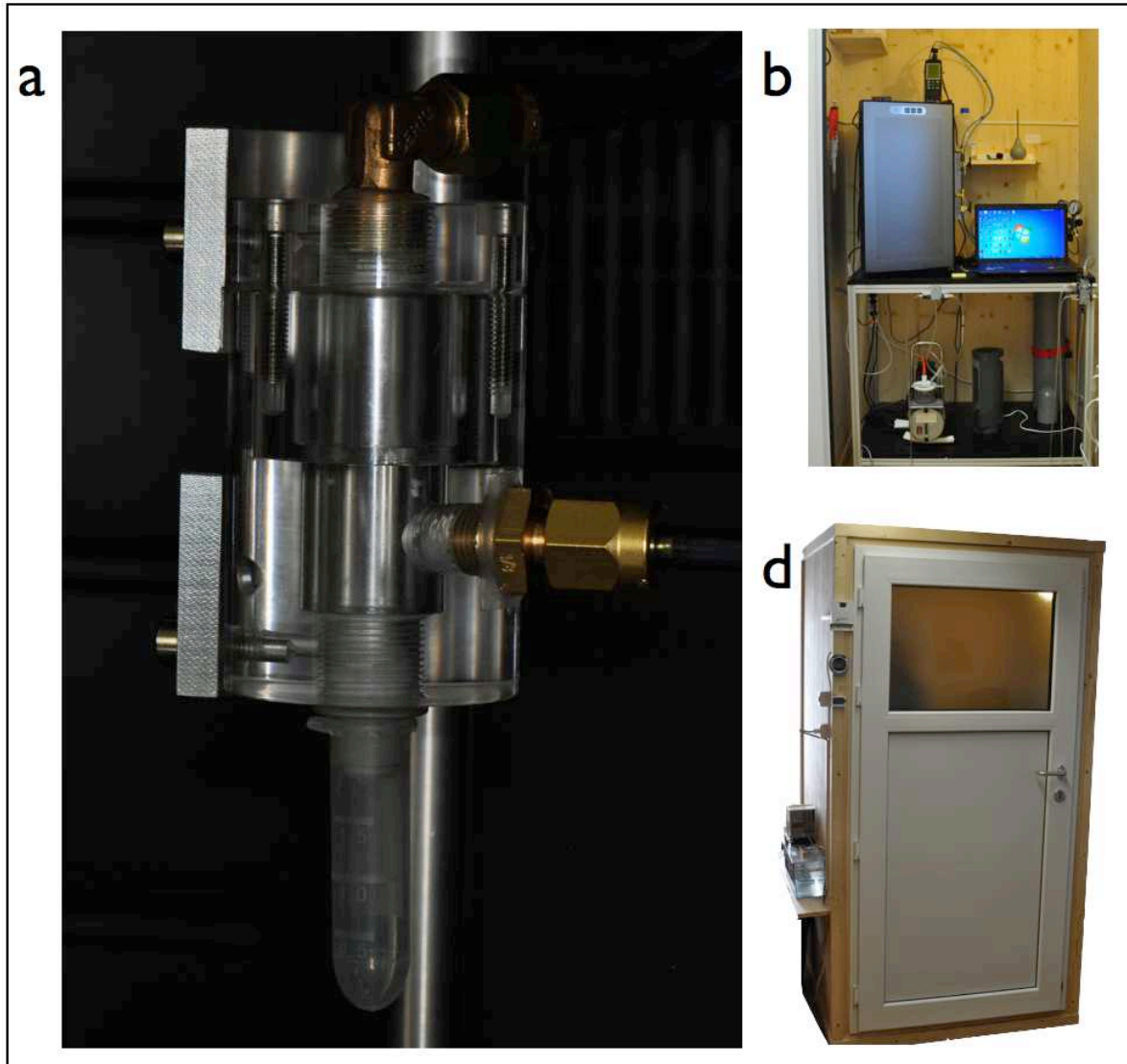


Fig 2: Stepwise temperature control; a) Split chamber mounted in the testing inner isolation room. b) Inner Isolation chamber. c) Outer Isolation room.

Figure 3:

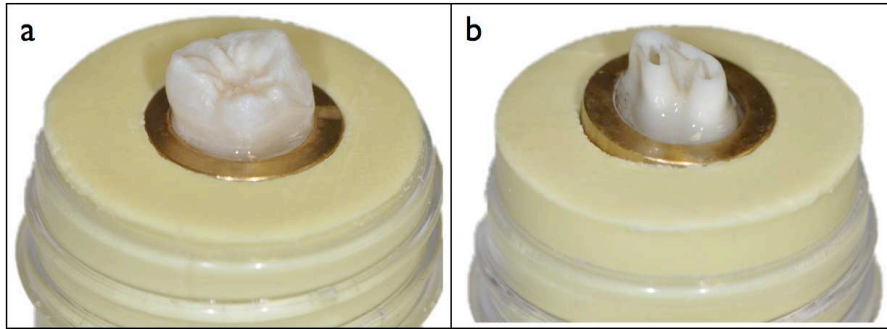


Fig 3: a) Embedding from coronal side, b) Embedding from apical side

Figure 4:

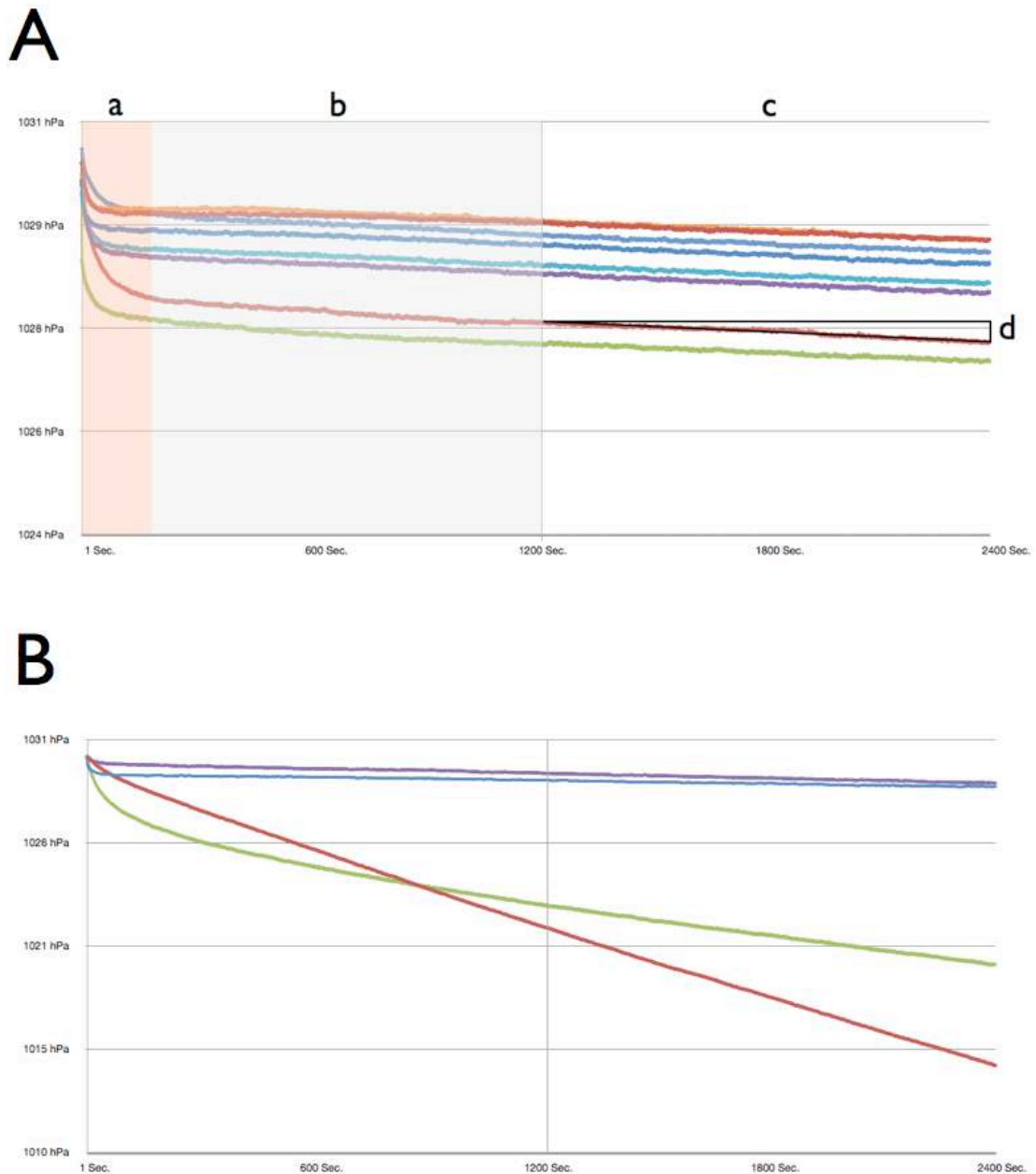


Fig 4: – Exp. A: A representantative graph of a tested sample with 8 repeated measurements for its baseline permeability (hPa/min): (a) The gas compensation curve (each pressurized gas will behave unstable for a period of time). (b) System stabilization curve, which is related to temperature compensation. (c) The permeability curve which is related to the sample permeability status. (d) The permeability slope.

- Exp. B: A representantative graph showing the permeability curves of a sample tested for multiple treatments. ■ Baseline curve. ■ After CI I preparation. ■ After full occlusal preparation. ■ After Cerec onlay restoration.

Figure 5:

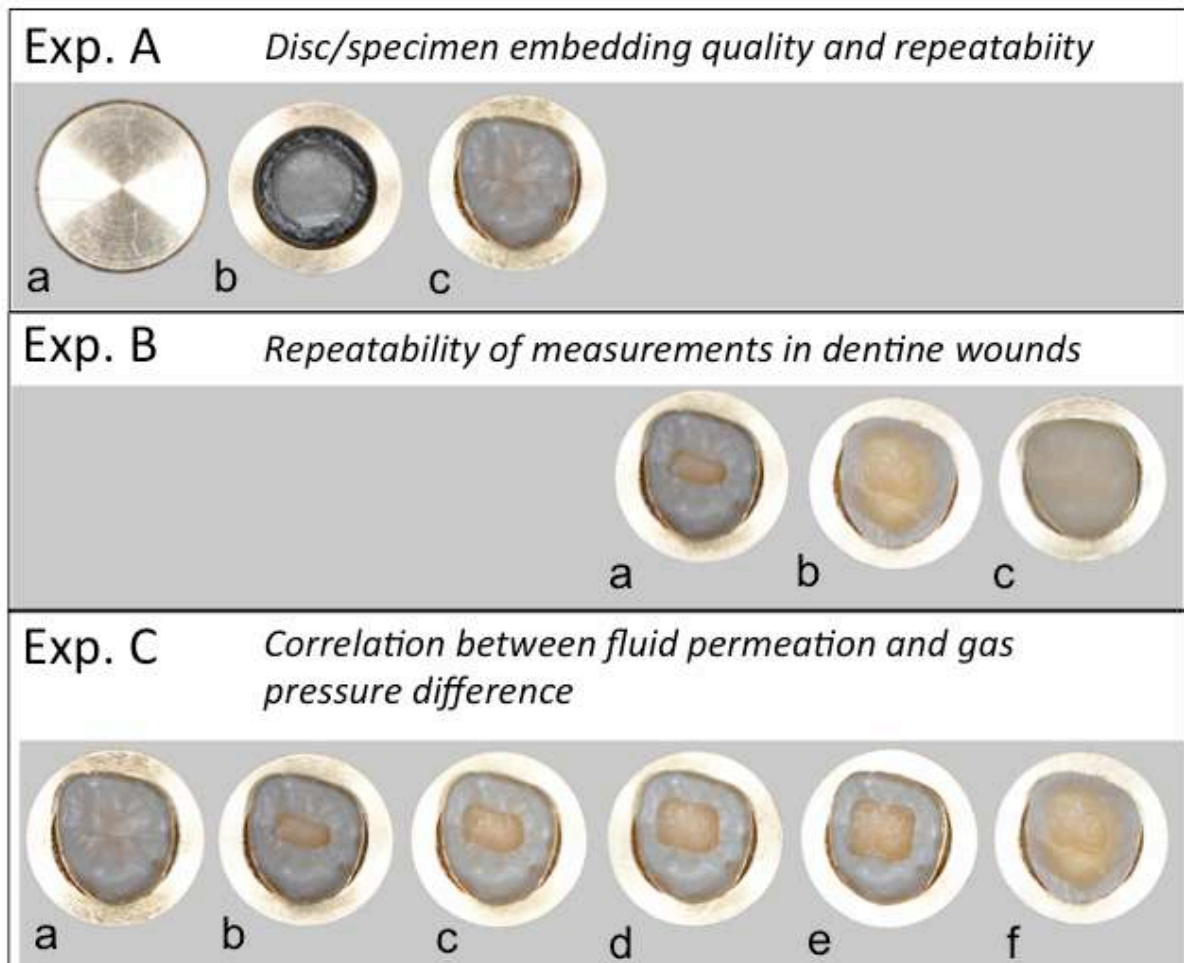


Fig 5: - Exp. A: Disc/specimen embedding quality and repeatability; One full metal disc (a, no embedding), three embedded composite discs (b) and three embedded third molars (c); eight consecutive measurements in each sample

- Exp. B: Repeatability of measurements in dentine wounds; Three molars (of Exp. A) with 2x5 mm (a) and full occlusal preparation (b) as well as consecutive restoration (c); eight consecutive measurements in each sample
- Exp. C: Correlation between fluid permeation and gas pressure difference; Six third molars (a) with step-wise increasing preparation size of 2x5, 3x5, 4x5, 5x5 mm (b-e) and full preparation (f); one measurement per sample.

Figure 6:

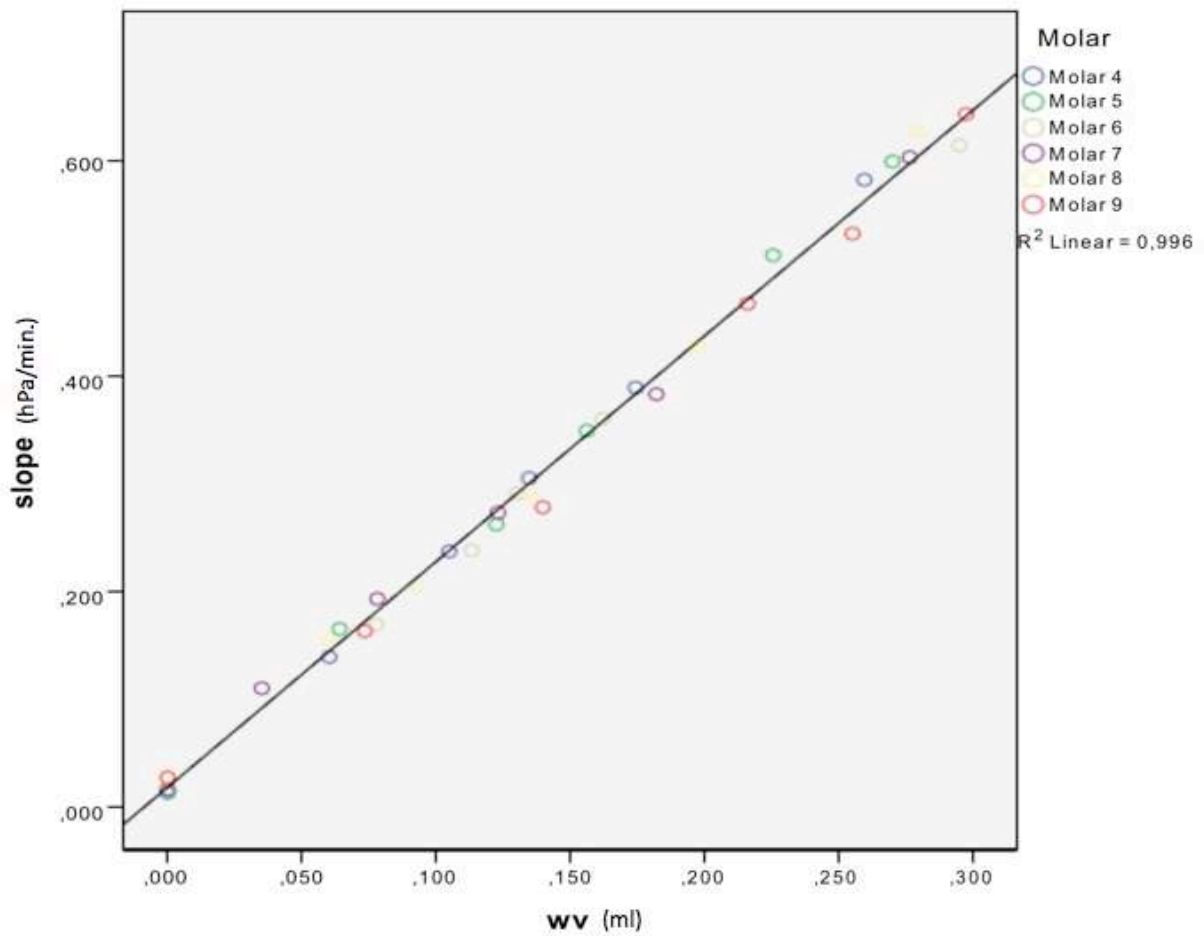


Fig 6: Plotted linear regression line, showing the correlation between the slope value (y-axis, denoted as Slope, measurement unit: hPa/min), and the permeated saline volume (x-axis, denoted by wv, measurement unit: ml